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available at www.sciencedirect.comwww.elsevier.com/locate/brainres**BRAIN
RESEARCH****Research Report****Gender differences in hyperthermia and regional 5-HT and 5-HIAA depletion in the brain following MDMA administration in rats**

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ABSTRACT

In the present research the role of gender in MDMA-induced hyperthermia and serotonin depletion is studied by injecting male and female male rats with MDMA or saline 3 times (i. p.) with 3 h interval at dosages of 0.3, 1, 3 or 9 mg/kg at an ambient temperature of 25 °C. The acute hyperthermia following the higher dosages was much stronger in males than in females. After the highest dose, body temperature was even raised for several days. This effect was particularly present in males where nocturnal hyperthermia persisted the whole 4-week period of sampling. Despite the differences in the acute hyperthermic response, no significant gender differences were found in 5-HT depletion 4 weeks after MDMA (9 mg/kg) administration. A striking difference was present, however, in the concentration of the 5-HT metabolite 5-HIAA after MDMA administration. In males 5-HIAA levels decreased, whereas in females this metabolite was hardly affected, suggesting a lasting increase in 5-HT turnover in females following drug administration. When genders were matched for their acute physiological hyperthermic response by repeated injection of 9 mg/kg in female rats and 6 mg/kg in male rats, 5-HT depletion was only present in females. In this experiment with matched acute physiological responses 5-HIAA levels also decreased much stronger in males, suggesting an increased 5-HT turnover in females 4 weeks after MDMA administration. In conclusion, although male rats are clearly more susceptible for the acute as well as the lasting hyperthermic effects of MDMA than females, this is not reflected in levels of 5-HT depletion following administration of similar dosages of the drug. This may indicate that, in case of a similar thermogenic response, females have a higher 5-HT neurotoxicity following MDMA than males.

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1. Introduction

The popularity of the psychostimulant ecstasy (3,4-methylenedioxymethamphetamine; MDMA) has increased during

the last decades. One of the acute dangers of ecstasy (ab)use is the occurrence of a hyperthermia (for example see Dafters, 1994; Freedman et al., 2005; Malberg et al., 1996; Nash et al., 1988; O'Shea et al., 1998; Schmidt et al., 1990), which may lead

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to lethality (Chadwick et al., 1991; Dowling et al., 1987). Another reason for concern is the long-lasting, possibly neurotoxic consequences of MDMA for the serotonergic system. Several preclinical studies have demonstrated that MDMA evokes a persistent decrease in serotonin and 5-hydroxyindoleacetic acid (5-HIAA), a reduction in tryptophan hydroxylase (TPH) activity, a reduction of serotonin transporter (SERT) activity and expression and finally a long-term impairment of anterograde transport in serotonin axons (Battaglia et al., 1987; Buchert et al., 2004; Callahan et al., 2001; Colado et al., 1993; Hewitt and Green, 1994; Ricaurte et al., 2000; Schmidt and Taylor, 1987; Semple et al., 1999; Sharkey et al., 1991; Stone et al., 1986; Xie et al., 2006). Although clinical studies on this matter are less conclusive, there is increasing evidence that MDMA can be toxic for the human brain too. (de Win et al., 2004; Grob, 2002; McCann et al., 2000; Turner and Parrott, 2000).

In preclinical psychopharmacological research females are often not included because of the hormonal fluctuations due to the estrous cycle. However, preclinical and clinical studies emphasize the importance of sex differences in the pharmacokinetic and pharmacodynamic responses to many drugs of abuse, including alcohol, nicotine and psychostimulants like cocaine and amphetamine (Becker et al., 2001; Brady and Randall, 1999; Carroll et al., 2004; Lynch et al., 2002).

In view of the fact that the use of ecstasy increased significantly among females together with the high popularity of ecstasy (Allott and Redman, 2007) it is important to consider gender as a modulatory factor in the long-lasting effects of MDMA on serotonergic functioning. Therefore, the present study aimed at the question of gender differences in the long-lasting serotonergic neurotoxic consequences.

Data from studies in humans do suggest a gender difference. Females seem to be more susceptible than males to deficiencies in 5-HT functioning after ecstasy use (Buchert et al., 2004; Croft et al., 2001; McCann et al., 1994; Reneman et al., 2001). Furthermore, it was shown that equal doses of MDMA per kilogram body weight produced stronger subjective effects in women compared to men (Liechti et al., 2001).

So far, animal studies have found no (consistent) difference between male and female rats in depletion of 5-HT and 5-HIAA levels after MDMA administration (Chu et al., 1996; Koenig et al., 2005; Walker et al., 2007). Regarding gender differences in MDMA-induced changes in body temperature and behavioral locomotor response, conflicting results are reported (Colado et al., 1995; Fonsart et al., 2008; Koenig et al., 2005; Palenicek et al., 2005; Walker et al., 2007; Wyeth et al., 2009).

The present study investigated whether male and female rats differ in the acute hyperthermic and long-term body temperature regulation in response to MDMA administration and in the 5-HT depleting effects of the drug. The study was divided into two experiments. In the first experiment it was investigated whether there are differences in acute and long-term body temperature effects and in the long-term serotonergic depletion between male and female rats after MDMA administration. Different dosages of MDMA were administered and the MDMA-induced hyperthermic response was measured in both males and females. Body temperature was measured using biotelemetry, to assure body temperature sampling in a stress-free manner. To control for interaction effects with body temperature, in a second experiment male

and female rats were matched for their acute MDMA-induced hyperthermic peak response and it was investigated whether these male and female rats differed in the long-term serotonin depletion.

2. Results

2.1. Experiment 1

2.1.1. Acute effect of MDMA on body temperature

In Fig. 1a the AUC is plotted for the temperature response between 0 and 9 h after three injections of MDMA in different doses. MDMA induced a significant hyperthermic response in rats administered 3×9 mg/kg MDMA (main dose effect $F(4,47) = 30.954$, $p < 0.0001$). When taking also gender into account, the two-factor ANOVA revealed a significant gender \times dose interaction effect ($F(4,47) = 8.388$, $p < 0.0001$). The dynamics of the temperature response in male and female rats following administration of the highest dose is visualized in Fig. 1b. As shown in Fig. 1a and b, the hyperthermic response was significantly stronger in male than in female rats ($t(9) = -4.990$,

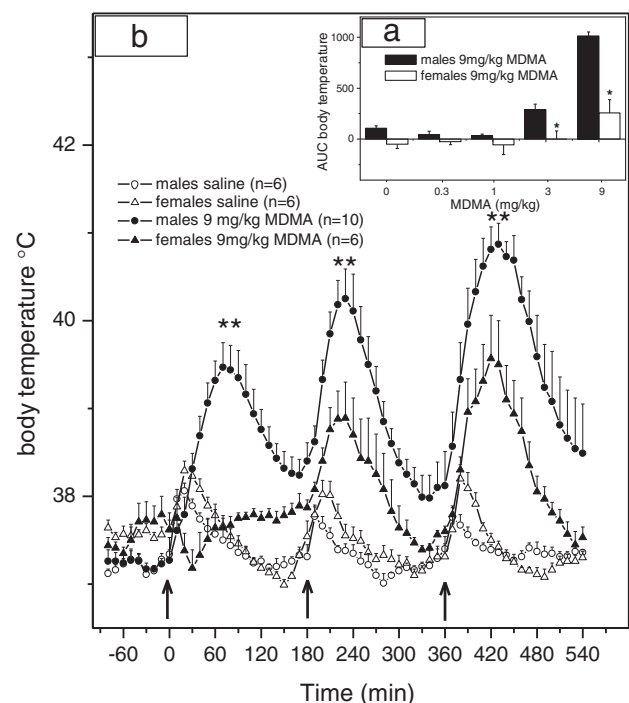


Fig. 1 – (a) Average total body temperature (\pm SEM) response measured as area under the curve (AUC) over the first 9 h after the first MDMA injection. Male and female rats were injected 3×0.3 , 3×1 , 3×3 or 3×9 mg/kg MDMA or saline.

*** $p < 0.05$, difference between males and females treated with similar doses. (b) Average body temperature (\pm SEM) of male and female rats over time. Rats were injected three times with 0 or 9 mg/kg MDMA. Injections were administered at time points 0, 3 and 6 h. Arrows indicated time of injection. ** $p < 0.01$, comparison between male and female peak responses following 3×9 mg/kg MDMA.**

$p < 0.001$). The dose of 3×3 mg/kg MDMA only induced a significant hyperthermic response in males ($F(4,23) = 112.179$, $p < 0.0001$).

2.1.2. Lethality

Since the highest dose (3×9 mg/kg) elicited a strong hyperthermic response in particular in male rats this was coinciding with a high lethality in the male animals. Out of 15 male rats being administered with 3×9 mg/kg only 6 survived (survival rate 40%), while out of 9 female rats injected with 3×9 mg/kg 8 rats survived (survival rate 89%). Employing logistic regression using the model $Y = \text{LOG}(p/(1-p)) = ax + b$ (p = chance of a rat dying and a zero model was used for fit, with $y = \text{constant}$) revealed that dosage ($X^2 = 30.88$, $p < 0.0001$) of MDMA and gender ($X^2 = 6.13$, $p < 0.05$) together determined survival rate, indicating that significantly more males than females died after receiving 3×9 mg/kg MDMA.

2.1.3. Long-term effect on body temperature

Also on the long-term, MDMA treatment resulted in a differential body temperature response in male and female rats. A significant main effect of gender ($F(1,19) = 8.0$; $p = 0.01$) and treatment ($F(1,19) = 12.9$; $p = 0.002$) was present in temperatures during the day, although there was no significant interaction between gender and treatment. Comparison of the night temperatures did show, however, a significant interaction between gender and treatment ($F(1,19) = 16.9$; $p = 0.001$). Post hoc analysis revealed that female rats receiving 3×9 mg/kg MDMA were hyperthermic during the light phase 1 day after repeated MDMA treatment, whereas male rats stayed hyperthermic during the light phase until day 3 after MDMA administration (see Fig. 2). MDMA treatment of 3×9 mg/kg in male rats increased body temperature during the dark phase (active period of the day) for the whole registration period as compared to female rats treated with the drug. Actually MDMA in 3×9 mg/kg dose tended to suppress body temperature during the night in the female rats.

2.1.4. Monoamine concentrations

Monoamine concentrations were measured in the following 8 brain regions: prefrontal cortex, hippocampus, parietal cortex, septum, cerebellum, brainstem, hypothalamus and striatum. The direction of changes was similar in all brain regions, although local differences existed in the magnitude of the observed changes. For an easy interpretation of the data we therefore restricted the graphical visualization of the data to only 3 brain regions: prefrontal cortex (PFC), hippocampus and striatum.

MDMA (3×9 mg/kg) clearly induced 5-HT depletion in these three brain areas (main treatment effect, $F(3,26) = 27.6$; $p < 0.001$). There was an effect of gender ($F(3,26) = 2.9$; $p = 0.052$) but male and female rats did not significantly differ in the induced 5-HT depletion (no significant treatment \times gender interaction (Fig. 3a).

MDMA evoked a depletion in the serotonergic metabolite 5-HIAA in all three brain areas (main treatment effect, $F(3,26) = 28.1$, $p < 0.001$). When gender was also taken into account, a significantly larger 5-HIAA depletion was found in the hippocampus of male rats compared to female rats (treatment \times gender interaction ($F(1,28) = 7.9$, $p < 0.01$) (Fig. 3b). Post

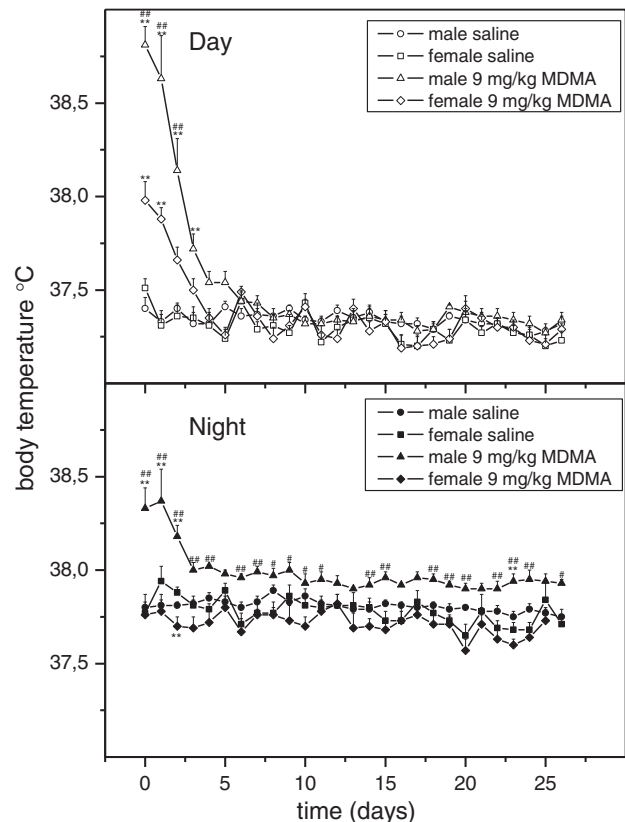


Fig. 2 – Average body temperature (\pm SEM) of male and female rats per 12 h during 28 days. At day zero repeated MDMA (3×9 mg/kg) or saline injections were given. Light symbols in the upper graph represent the average body temperature during the light phase. Dark symbols in the lower graph represent the average body temperature during the dark phase. ** $p < 0.01$, comparison with saline treated controls; # $p < 0.05$, ## $p < 0.01$, comparison between MDMA treated males and females.

hoc testing showed that 5-HIAA levels in the prefrontal cortex and hippocampus were significantly higher in MDMA treated females than in MDMA treated males ($p < 0.05$ and $p < 0.001$, respectively).

MDMA did not induce a depletion of DA, DOPAC, HVA and NA levels in any of the measured brain areas (data not shown).

2.2. Experiment 2

The results of experiment 1 showed that MDMA induced a significantly higher acute hyperthermia in males than in females and that this did not coincide with sex differences in serotonin depletion in the brain. As explained in the [Experimental procedures](#), in a subsequent experiment we aimed to match males and female for their hyperthermic response to MDMA and investigated the long-term effect on the serotonergic system in the brain. For this purpose we added a group of male rats that were injected with 3×6 mg/kg MDMA. This dose in males induced a hyperthermia that matched the

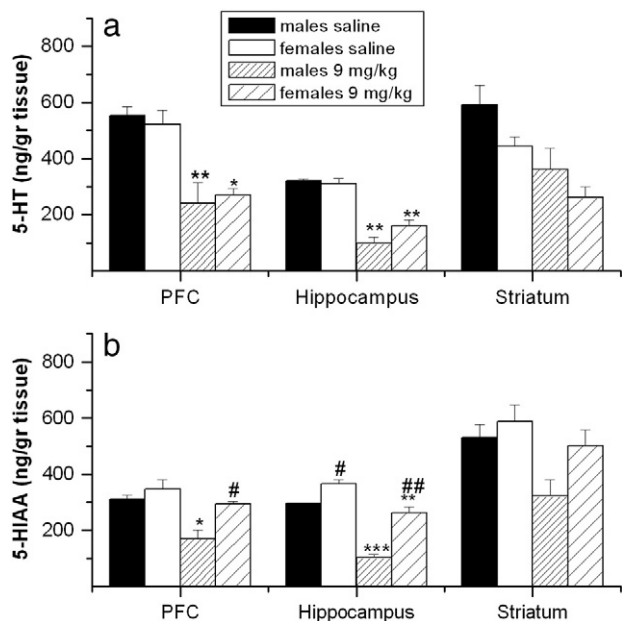


Fig. 3 – Average (a) 5-HT and (b) 5-HIAA tissue concentration (±SEM) as measured in prefrontal cortex (PFC), hippocampus and striatum of male and female rats, 4 weeks after repeated saline or MDMA injections (3 × 9 mg/kg). * $p < 0.05$, ** $p < 0.01$, comparison between MDMA treated rats and vehicle treated controls; # $p < 0.05$, ## $p < 0.01$, comparison between MDMA treated males and females.

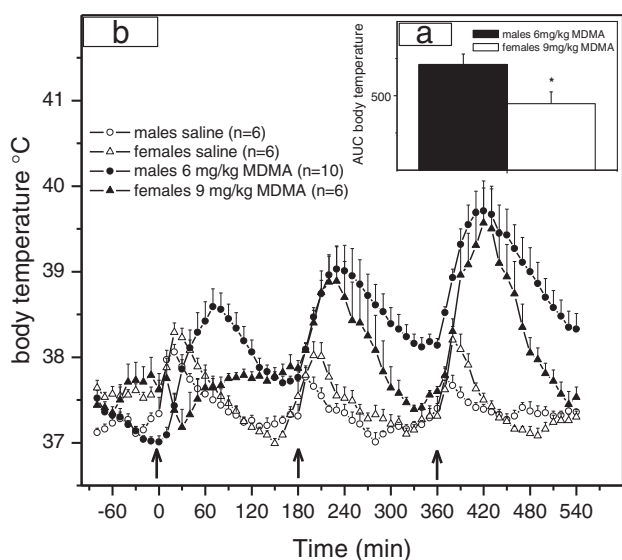


Fig. 4 – (a) Average total body temperature (±SEM) response measured as area under the curve (AUC) over the first 9 h after the first MDMA injection. Female rats were injected three times with 0 or 9 mg/kg MDMA. Male rats were injected three times with 0 or 6 mg/kg. * $p < 0.05$, difference between males and females. (b) Average body temperature (±SEM) of male and female rats over time. Female rats were injected three times with 0 or 9 mg/kg MDMA. Male rats were injected three times with 0 or 6 mg/kg. Injections were administered at time points 0, 3 and 6 h. Arrows indicated time of injection.

hyperthermia in female rats that were injected with 3 × 9 mg/kg MDMA as indicated in Fig. 4. Although the AUC measurements (see Fig. 4a) of the temperature following drug administration show that in male rats, even with a lower dose of 6 mg/kg, the overall acute hyperthermic response was still larger than that in females after 9 mg/kg MDMA, no general gender difference existed in peak temperatures (Fig. 1b). Since in experiment 1 MDMA only resulted in a depletion of 5-HT and 5-HIAA concentrations, DA, DOPAC, HVA and NA concentrations were not analyzed.

2.2.1. 5-HT and 5-HIAA analysis

Statistical analysis revealed that MDMA induced a significant depletion in 5-HT concentrations in the three brain areas (main treatment effect, $F(3,30) = 10.6$, $p < 0.001$). Similar to experiment 1, the magnitude of the 5-HT depletion did not differ between both genders (Fig. 5a). Post hoc analysis revealed that MDMA induced only in females a 5-HT depletion in the PFC and hippocampus (Fig. 5a).

MDMA induced also a 5-HIAA depletion (main treatment effect $F(3,30) = 22.3$, $p < 0.001$). There also was a main effect of gender ($F(3,30) = 12.7$; $p < 0.001$). Post hoc analysis revealed that 5-HIAA significantly dropped in all male brain regions after 6 mg/kg MDMA and that in the prefrontal cortex and hippocampus 5-HIAA levels were also significantly lower than in females treated with 9 mg/kg MDMA (Fig. 5b).

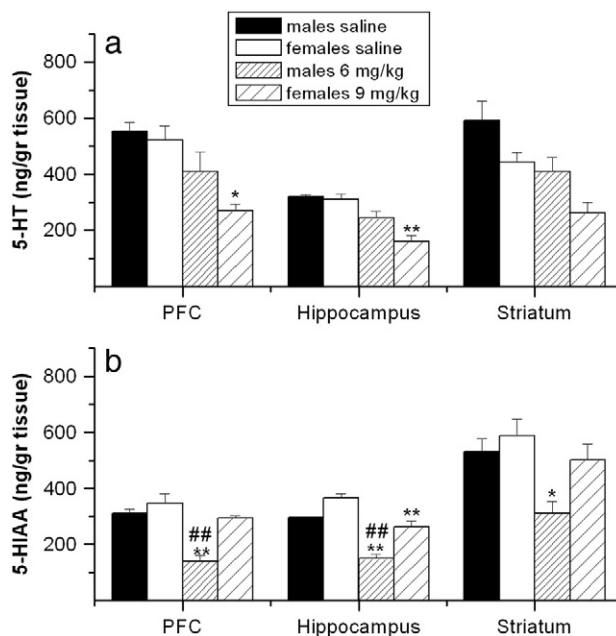


Fig. 5 – Average (a) 5-HT and (b) 5-HIAA tissue concentration (±SEM) as measured in prefrontal cortex (PFC), hippocampus and striatum of male and female rats, 4 weeks after repeated saline or MDMA injections (3 × 9 mg/kg in female rats and 3 × 6 mg/kg in male rats). * $p < 0.05$, ** $p < 0.01$, comparison between MDMA treated rats and vehicle treated controls; ## $p < 0.01$, comparison between MDMA treated males and females.

3. Discussion

The present study aimed to reveal gender differences in the acute and lasting effects on temperature regulation and monoamine depletion after MDMA treatment, using several dosages of MDMA. MDMA induced a much stronger acute hyperthermic response in male rats compared to female rats following treatment with 3×9 mg/kg MDMA which coincided with a higher lethality rate. This indicates that male rats are more vulnerable for the acute hyperthermic and lethal effects of MDMA than female rats. Our finding is in agreement with previous studies (Fonsart et al., 2008; Koenig et al., 2005; Wyeth et al., 2009). Furthermore, the lasting increase in body temperature during the night time that was observed only in male rats receiving the highest dose of MDMA indicates that male rats are also more vulnerable for long-lasting changes in temperature regulating mechanisms than female rats.

It is well known that the MDMA-induced long-term depletion of 5-HT and 5-HIAA tissue concentrations is related to the presence of an acute MDMA-induced hyperthermic response (Dafters, 1994; Malberg and Seiden, 1998). When the acute hyperthermia is absent, 5-HT depletion is abolished or attenuated (Broening et al., 1995; Colado et al., 1998; Farfel and Seiden, 1995; Malberg et al., 1996; O'Shea et al., 2002). Therefore, it was surprising to notice that, despite the differences in hyperthermic responses, male and female rat did not differ in the magnitude of the MDMA-induced 5-HT depletion. Another striking observation was that 5-HIAA depletion was only observed in males. It is well known that MDMA is able to inhibit MAO-A activity (Leonardi and Azmitia, 1994). It is possible that MDMA inhibits MAO-A enzyme activity to a greater extent in males than in females, inducing only a significant depletion of 5-HIAA concentrations in male rats. Furthermore, it can be hypothesized that due to gender differences in the 5-HT system in the medial preoptic nucleus (Simerly et al., 1984) which possibly includes MAO-A enzyme activity, that MDMA administration induces a stronger inhibition of MAO-A activity causing a higher extracellular 5-HT release in male rats and therefore inducing a greater hyperthermic response. Another possible explanation for the higher hyperthermic response in males may be found in the effects of the metabolite of MDMA, MDA, which causes a stronger hyperthermic peak response in males than in females (Fonsart et al., 2008). Since MDMA seems to be metabolized stronger in male than in female rats resulting in higher MDA levels in the male rats (Fonsart et al., 2008), the higher plasma MDA levels might play an important role in the stronger hyperthermia in male rats.

Since the temperature response differed so strongly between the genders we added a group of male rats receiving a lower dose of MDMA that would match the peak temperatures reached in females using the highest dose. In female rats having similar peak temperature responses as males, 5-HT decreased significantly, whereas concentration of 5-HT was not significantly decreased in males.

There is a circadian rhythmicity in body temperature regulation with temperatures being higher during the active period of the day (which is the dark phase for nocturnal animals like rats) and lower during the inactive period (light

phase) of the day. For several days after MDMA treatment, particularly in males the body temperature is higher in the light phase than in the dark phase. This might indicate that MDMA induces a temporal shift in circadian rhythmicity. Indeed, there is evidence indicating that MDMA can induce abnormalities in sleep and circadian patterns (Balogh et al., 2004; McCann and Ricaurte, 2007) in humans and rodents.

A number of points for consideration come up with regard to the performed experiment. Firstly, mortality rates show that 2/3 of the males died after receiving 3×9 mg/kg MDMA. This may have caused a bias in the interpretation of the outcome of the monoamine analysis. It is possible that the male rats that died after the MDMA injections are the rats that would also have the largest 5-HT depletion. The surviving rats might be relatively resistant to the MDMA-induced 5-HT depletion. If this would be true, it would result in an underestimation of the serotonergic depletion in males that received 3×9 mg/kg MDMA and a biased conclusion regarding the possible gender differences in vulnerability for MDMA-induced neurotoxicity. Importantly, after administration of 3×6 mg/kg MDMA to male rats all rats survived, resulting in an unbiased gender comparison.

Another point of concern may be the difference in body weight between males and females and sex-linked differences in pharmacokinetics. In general adult male rats are heavier than females (in the present study about 160 g difference). Since MDMA is administered in a dose per kg body weight the injected total amount of MDMA is therefore higher in males than in females. There are, however, extensive pharmacokinetic studies available comparing gender effects in male and female rats (Chu et al., 1996; Fonsart et al., 2008; Fonsart et al., 2009; Hirt et al., 2010). These studies all clearly indicate that conversion of MDMA to the metabolite 3,4-methylenedioxymphetamine (MDA) is significantly higher in male than in female rats which is probably due to higher activity of enzymes involved in N-demethylation of MDMA in males (Fonsart et al., 2008; Hirt et al., 2010). Females apparently are more exposed to MDMA and males to MDA and consequently to the metabolite 4-hydroxy-3-methoxyamphetamine (HMA) (Hirt et al., 2010). Since it has been hypothesized that these metabolites play a particularly important role in the neurotoxicity of the drug this would render male rats more vulnerable to the 5-HT depleting effects of MDMA which is not confirmed in our study. The present data do confirm findings in previous studies that toxic lethality is higher in males (Fonsart et al., 2008) due to an increased hyperthermia. Recent evidence in humans supports the existence of a gender difference in the thermogenic response. Females seem to be less vulnerable for the induction of a hyperthermic response by MDMA due to differences in their thermoregulatory abilities (Wyeth et al., 2009). Considering the fact that MDMA is metabolized more rapidly in males as mentioned above and the knowledge that the hyperthermic response following MDMA is positively correlated with 5-HT depletion (Malberg and Seiden, 1998), it is surprising to see in the present study that there is no increased 5-HT depletion in males on the long term which confirms earlier data in rats (Chu et al., 1996). Human studies even suggest that females might be more vulnerable to deficiencies in 5-HT functioning after ecstasy use (Reneman et al., 2001). Our data suggest that with similar

acute physiological responses female individuals show a slightly stronger 5-HT depletion in some brain areas than males. Since with ecstasy consumption male and female users consume similar dosages per pill, females may be exposed to higher dosages per kilogram body weight since males are in general heavier than females.

In conclusion, males seem to be more at risk of developing an acute, potentially lethal, MDMA-induced hyperthermic response than females. Our data indicate that 5-HT depletion in males and females with similar hyperthermic responses tends to be stronger in the female individuals. This supports the hypothesis that under conditions of a similar thermogenic response females are more vulnerable to the 5-HT neurotoxicity of MDMA.

4. Experimental procedures

4.1. Animals and housing

All experiments have been approved by the animal experiments committee of the University of Groningen. Male and female wild-type Groningen rats were used. Their ancestors were originally wild-trapped animals, subsequently bred in our laboratory for more than 31 generations. Females were screened for the stage of the estrous cycle they were in. The rats were in different stages and clearly not synchronized. The results showed, however, that the magnitude of the standard deviations in the outcome of the physiological as well as biochemical data was not different between male and female animals. This indicates that different stages of the estrous cycle are not having a major impact on the acute and lasting effects of MDMA. Room temperature was 21 °C, except during MDMA administration. Males and females were housed in separate rooms. All rats were individually housed in clear Perspex cages (31×15×14 cm) with sawdust bedding in a room with 12:12 light:dark cycle (lights on at 8:00 h). Food (chow) and water was available ad libitum throughout the whole experiment.

4.2. Experiment 1

In the first experiment 45 female and 50 male wild-type Groningen rats (*Rattus norvegicus*) of 10 weeks old (body weight: male 370±3.8 g, females 210±2.2 g at the moment of MDMA injection) were used.

4.2.1. Telemetry

A radio-telemetry system (Data Sciences International, St. Paul, MN) was used for stress-free monitoring of body temperature. Fourteen days prior to MDMA administration transmitters (TA10TA-F40; sensitivity: 0.1 °C) were implanted intraperitoneally (i.p.) in 28 males and 30 females using O₂-isoflurane anaesthesia. Temperature was measured every 10 min throughout the whole experiment. The cages of the rats were placed on receiver plates and the signal was collected and analyzed using the DSI Dataquest® A.R.T. Acquisition System.

To determine the long-term effect of MDMA on body temperature, data averages of 12 h (matching the light and the dark period) were calculated.

4.2.2. MDMA/saline injections

3,4-Methylenedioxymethamphetamine (± MDMA-HCl, 99.6%, obtained from the Dutch Forensic Institute, The Netherlands) was injected (i.p.) three times with intervals of 3 h ("binge administration") during the light phase. The first injection was given 1.5 h after lights went on. MDMA was given to both females and males at concentrations of 0.3, 1, 3 and 9 mg/kg MDMA, dissolved in 1 ml ultra purified water. Control animals were saline injected. Ambient temperature was 25 °C (± 0.5), starting from 1.5 h before the first injection until 3 h after the last injection.

4.2.3. Analysis of brain monoamine concentrations

It has been demonstrated that MDMA-induced 5-HT depletion only occurs when acutely a significant hyperthermic response is induced (Green et al., 2003). Therefore, long-term effects of MDMA administration on monoamine concentration in brain areas were only measured in male and female rats receiving the high dose of MDMA, i.e., 3×9 mg/kg, since this dose clearly elicited a hyperthermic response.

Four weeks after treatment these male and female rats were decapitated under brief (30 s) CO₂ anesthesia in the early light phase. From these animals, prefrontal cortex (PFC), striatum, hippocampus, cerebellum, brainstem (Bregma –8.50 mm to 16 mm), hypothalamus, parietal cortex and septum were dissected on a chilled plate, immediately snap frozen in liquid nitrogen and stored at –80 °C. For determination of 5-HT, 5-HIAA, dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and noradrenalin (NA) in these areas, high performance liquid chromatography (HPLC) was used. For this, samples were homogenized in 0.5 ml 0.1 M perchloric acid and centrifuged at 14000 rpm for 10 min at 4 °C. Supernatant was removed and analyzed for 5-HT, 5-HIAA, DA, DOPAC, and NA concentrations. For determination of the monoamines and their metabolites, 100 µl was injected onto a reversed phase Gemini C18 column (150×4.6 mm, 5 µm particle size), connected to a detector (ESA coulochem model 5100A) with a 5011A detector cell. A difference in potential of 340 mV was set (the potential of one electrode was 0 mV and the other was 340 mV). The mobile phase consisted of 62.7 mM Na₂HPO₄, 40.0 mM citric acid, 0.27 mM EDTA, 4.94 mM HSA, 10% methanol at pH 4.1 with a flow of 0.5 ml/min. Known amounts of 5-HT, 5-HIAA, DA, DOPAC, HVA, (Sigma Chemicals) and NA (Research Biochemicals International) were run throughout the whole procedure for standardization. Monoamine levels were calculated as ng/g tissue.

4.3. Experiment 2

The second experiment was performed to control for possible difference in serotonergic depletion between genders due to a difference in their MDMA-induced hyperthermic response. Therefore, in this experiment an additional group of 10 male rats (body weight 364±8.7 g at the day of MDMA administration) was injected with a dose of MDMA that matched the peak temperature response in the female rats in experiment 1 that received 9 mg/kg MDMA. We chose to match males to the hyperthermic response of females that received 3×9 mg/kg MDMA in the first experiment, instead of matching females to the hyperthermic response of males

that were receiving 3×9 mg/kg MDMA, since a hyperthermic response of this magnitude apparently holds a high risk of lethality.

When males were injected with 3×6 mg/kg MDMA the acute hyperthermic peak response following each injection appeared to be similar to that in females injected with 3×9 mg/kg MDMA. The timing and methods of implantation of telemetry transmitters, drug administration and brain monoamine analysis were all similar as described previously under experiment 1.

4.4. Statistical analysis

SPSS 14.0 for Windows was employed to analyze the data statistically. Acute body temperature response (area under the curve (AUC)) was analyzed using a two-factor ANOVA with treatment (5 levels) and gender (2 levels) as between-subject factors. Peak temperatures in the acute body temperature response were compared using ANOVA with repeated measures with 3 peaks following the injections (3 levels within subject) and gender (2-levels) as between-subject factor. The long-term body temperature was analyzed using repeated-measurements ANOVA with treatment (5 levels) and gender (2 levels) as between-subject factors and was tested as the within-subject repeated measurements factor. In case of significant main effects, post hoc analyses were performed using a Bonferroni test to reveal the differences between both genders for each dose. A one-way ANOVA was used to reveal for each gender separately the differences between the several doses.

Lethality rate of males and females for each dose was statistically analyzed using logistic regression. In experiments 1 and 2, monoamine concentrations were statistically analyzed using a two-factor ANOVA with treatment (2 levels) and gender (2 levels) as between-subject factors. In case of a significant main effect post hoc analysis was performed using a Bonferroni test to reveal the differences between both genders for each dose.

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